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Effects of Suanzaorentang on Behavior Changes and Central Monoamines

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ABSTRACT

Previously, it was found that the ancient Chinese remedy of Suanzaorentang could be a promising anxiolytic drug (Chen and Hsieh, 1985a, Chen and Hsieh, 1985b). To understand the mechanism of the action of Suanzaorentang, the effects of Suanzaorentang on behavior changes and central monoamines and their metabolites were studied in rats. It was found that Suanzaorentang significantly (1) prolonged the period from the onset of clonic to tonic convulsions induced by pentylenetetrazol or picrotoxin, (2) prolonged the sleep duration induced by hexobarbital, (3) reduced locomotor activity, (4) enhanced the hypomotility induced by α-MT, (5) reduced the locomotor stimulation produced by levodopa plus benserazide, and (6) reduced central HVA, VMA, and 5-HIAA, but had no significant effects on central DA, NA, and 5-HT. These facts implied that Suanzaorentang decreased the turnover rate of central monoamines and central catecholaminergic activity.

Key words: Suanzaorentang, Monoamines.

Introduction

The ancient Chinese remedy of Suanzaorentang (S-T) was originally prescribed for patients with insomnia (Chang, 115 B.C.). In our clinical trials of this remedy, we found that in appropriate doses, it could be useful in patients with insomnia (Chen and Hsieh, 1985a) and it could be a very promising anxiolytic remedy (Chen and Hsieh, 1985b). It improved the mood, alleviated the accompanying sympathetic symptoms, and improved the psychomotor performance.

The purpose of the present study was to examine the mechanism of action of S-T. We studied the effects of S-T on drug-induced convulsions, drug-induced sleep, locomotor activity, central catecholaminergic activity, and the concentration of whole brain monoamines and their metabolites.

Materials and Methods

Preparation of S-T

The remedy of S-T is composed of 5 components: Zizyphi semen, Poria, Ligustici Chuanxiong Rhizoma, Anemarrhenae Rhizoma, and Glycyrrhizae Radix. The method of preparation of S-T has been described in our previous paper (Chen and Hsieh, 1985a). When given to rats, S-T was suspended in 0.5% CMC (carboxymethylcellulose).

Animals

Male Sprague-Dawley rats, weighing between 190 and 230 g, were used in the studies of locomotor activity. The animals were maintained in groups of 5 per cage at a controlled temperature of 22±1°C and humidity of 55± 5% and housed in a metal-wire mesh cage for at least 7 days before each experiment. A solid diet (Sin-Lon Co. Taichung) and tap water were provided ad libitum. Male Sprague-Dawley rats were also used for studying the concentration of whole brain monoamines and their metabolites. In studying drug-induced convulsions and drug-induced sleep, mice were used.

Drug and treatment regimens

The following compounds were used: α-methyl-p-tyrosine methylester HCl (α-MT, Sigma), levodopa (Kyowa Hakko Co. Japan), benserazide HCl (Hoffmann-La Roche), hexobarbital (Tokyo Hasei, Japan), pentylenetetrazol (Sigma), picrotoxin (Sigma), and strychnine (Sigma).

S-T was given p.o. Pentylenetetrazol, strychnine,

α-MT, levodopa, and benserazide were given i.p. Picrotoxin was given s.c. into the dorsal part of the neck.

S-T was given in dosages of 1.5, 3.0, or 6.0 g/kg (calculated as the weight of crude herb). Pentylenetetrazol (100 mg/kg), strychnine (1 mg/kg), and picrotoxin (8 mg/kg) were given 1 hr before S-T. Hexobarbital (80 mg/kg) was given 1 hr after S-T. The following compounds were given before the S-T as follows: α-MT (100 mg/kg) every 2 hr, levodopa (200 mg/kg) every 50 min, and benserazide (50 mg/kg) every 80 min. Control rats received 0.5% CMC i.p.

Effects of S-T on drug induced convulsions

The time period to produce clonic convulsion (C.C.) and the time period between clonic and tonic convulsions (T.C.) by gross observation were used as the indices of the effects of S-T on drug-induced convulsions.

Effects of S-T on drug induced sleep

Sleep time was recorded as the time period between the loss and the recovery of the righting reflex (Fuijmori, 1965).

Locomotor activity measurements

The locomotor activity of individual rats was measured, using the MK-Animex (Muromachi Kikai Co. Japan). The sensitivity and tuning of the instrument was adjusted to 35 μA to enable all kinds of motor behavior, including locomotion, rearing, grooming, sniffing, and licking to be jointly recorded. Activity was recorded for 60 min intervals for 5 hr, starting 5 min after inserting the animal into the test cage. Each rat was used only once (Hsieh, 1982).

Effects of S-T on the concentrations of whole brain monoamines-metabolites in rats

S-T was given orally to 3 groups of 5 rats in dosages of 1.5, 3.0, 6.0 g/kg respectively. Sixty min later, the rats were decapitated and the whole brain was removed and homogenized. The concentrations of serotonin, dopamine, norepinephrine, and their metabolites were measured by High Performance Liquid Chromatography with Electrochemical Detection (Gunther, 1982; Lackovic *et al.*, 1981; Shibuya *et al.*, 1982). HPLC Model M-45 (Waters Associates), Data Module Model M-730 (Waters Associates), and Electrochemical Detector Model LC-4B (Bioanalytical Systems) were used.

Table 1. Effect of S-T on Hexobarbital-Induced Sleep in Mice

Dose (g/kg)	Onset (min)	Sleep (min)
Control	8.41±0.50	37.70±2.79
1.5	7.72±0.46	51.00±2.91*
3.0	6.96±0.25	74.80±4.40*
6.0	6.57±0.22	85.10±6.96*

Onset: The time to produce disappearance of righting reflex
Sleep time: From the loss to the recovery of righting reflex
* $P < 0.05$ (compared to control)
n=10 mean ± S.E.

Statistical analysis

Results were analyzed statistically using analysis of variance to decide whether there is any significant difference between sets of data. If the difference is significant, then the comparisons were made between each experimental group and the control group using the studentized range test:

$$|\bar{X}n - \bar{X}o| = C\sqrt{\frac{MSE}{n}},$$

where $\bar{X}n$ is the mean value of each experiment group,
$\bar{X}o$ is the mean value of the control group,
C is the upper 5% value in the studentized range,
MSE is the mean square error, and
n is the number of data in each group.
A p value ≤ 0.05 was required for statistical significance.

Results

Effects of S-T on drug induced convulsions

The profile of giving pentylenetetrazol or picrotoxin was clonic convulsion, tonic convulsion, and then death. As shown in Table 2, S-T significantly prolonged the period from clonic to tonic convulsions by pentylenetetrazol ($P < 0.05$). Also shown in Table 3, S-T significantly prolonged C.C. and T.C. ($P < 0.05$) by picrotoxin. But S-T did not inhibit the strychnine-induced convulsion (Table 2).

Effects of S-T on hexobarbital induced sleep

Table 2. Effects of S-T on Pentylenetetrazol-, Picrotoxin-, and Strychnine-Induced Convulsions in Mice

Dose (g/kg)	Pentylenetetrazol		Picrotoxin		Strychnine	
	C.C. (min)	T.C. (min)	C.C. (min)	T.C. (min)	T.C. (min)	Time for death (min)
Control	0.61±0.03	3.03±0.28	6.23±0.17	5.67±0.41	2.48±0.22	2.82±0.25
1.5	0.64±0.03	4.06±0.42	6.22±0.25	5.41±0.45	2.25±0.23	2.65±0.22
3.0	0.66±0.03	5.04±0.37*	7.12±0.20*	6.31±0.26	2.35±0.20	2.91±0.19
6.0	0.66±0.03	6.19±0.52*	7.81±0.40*	8.85±0.42*	2.10±0.26	2.63±0.24

C.C.: The time to produce clonic convulsion. T.C.: From clonic to tonic convulsions. n=10. Mean ± S.E.
* $P < 0.05$ (compared to controls).

Table 3. Effect of S-T on Monoamines and Metabolites in Rats Brain

	Control (ng/g wet wt)	S-T 1.5 g/kg (ng/g wet wt)	S-T 3.0 g/kg (ng/g wet wt)	S-T 6.0 g/kg (ng/g wet wt)
NA	1647.9±206.1	1739.9±181.9	1674.6±149.9	1721.5±295.7
DA	1779.7±93.6	1760.6±69.7	1919.2±183.3	1953.2±334.1
5-HT	1775.2±278.8	1686.9±138.5	1756.5±118.6	1992.8±193.9
VMA	647.3±53.4	491.5±42.3	457.2±39.4*	293.3±51.2*
HVA	658.8±95.3	454.8±24.5	432.8±51.5	303.4±44.8*
5-HIAA	624.7±38.4	498.1±46.9	474.7±18.8*	319.7±46.6*

n=5, Mean ± S.E. * $P < 0.05$ (compared to control)

As shown in Table 1, S-T significantly ($P < 0.05$) potentiated the hypnotic effect of hexobarbital.

Effects of S-T on locomotor activity

Fig. 1 shows the results of the experiment in which different doses of S-T were administered to groups of 10 rats. S-T significantly decreased locomotor activity ($P < 0.05$).

As shown in Fig. 2, rats pretreated with α-MT appeared to be behaviorly depressed as compared with the 0.5% CMC control. The hypomotility produced by α-MT was significantly ($P < 0.05$) augmented by S-T.

As shown in Fig. 3, levodopa plus benserazide produced a significant increase in locomotor activity. In addition to this locomotor stimulation, stereotyped movements including head shaking, forepaw padding, jumping, sniffing, and licking were observed. Autonomic symptoms including salivation, exophthalmos, and piloerection were also seen.

As shown in Fig. 3, locomotor stimulation was strongly reduced by S-T ($P < 0.05$). And stereotyped movements, aggressive behaviors, and sympathetic symptoms were apparently decreased.

Effects of S-T on the concentrations of whole brain monoamines-metabolites

As shown in Table 3, monoamines (NA, DA, and 5-HT) were not significantly lowered. But their metabolites (VMA, HVA, and 5-HIAA) were significantly lowered ($P < 0.05$).

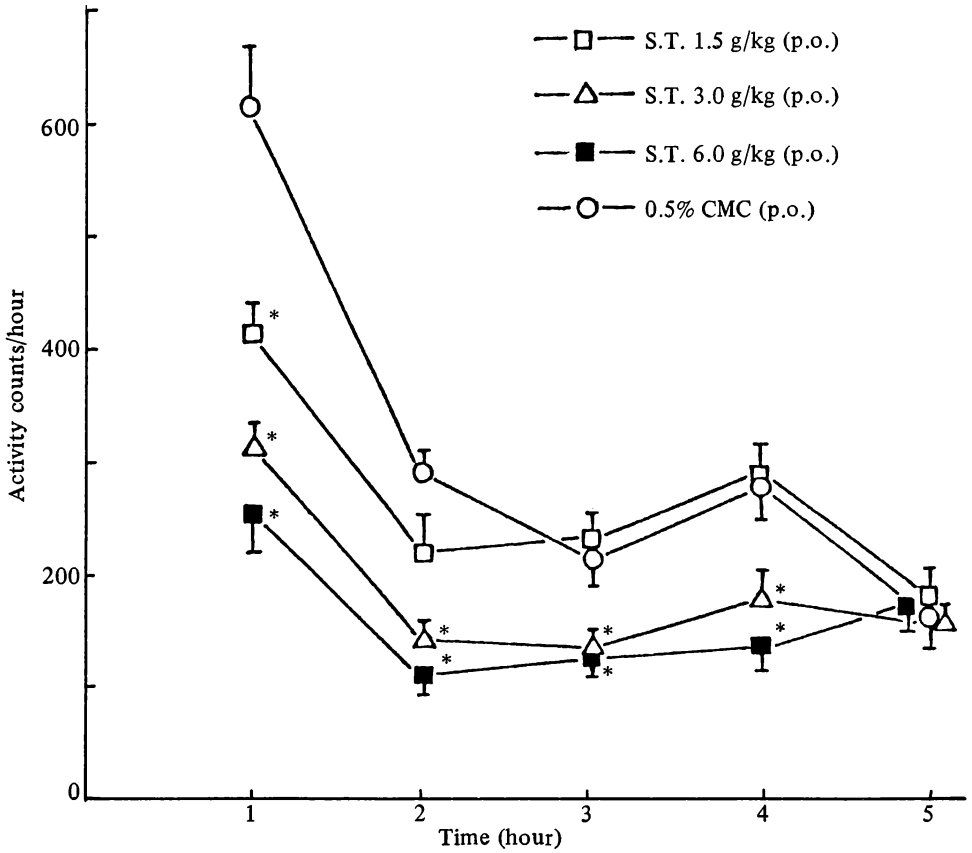

Fig. 1. Effects of Suanzaorentang on the locomotor activity in rats. Each point represents the mean of 10 independent observations. The vertical bars designate the S.E.s'. * $P < 0.05$ (compared to 0.5% CMC).

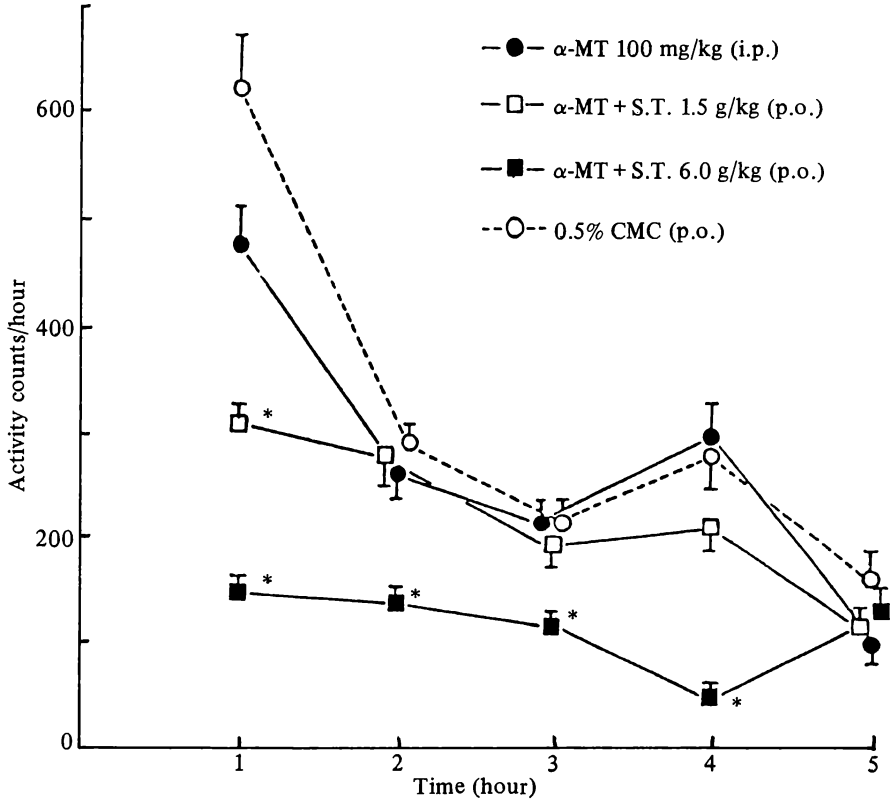

Fig. 2. Effects of Suanzaorentang on changes in locomotor activity produced by α-MT in rats. Each point represents the mean of 10 independent observations. The vertical bars designate the S.E.s'. * $P < 0.05$ (compared to α-MT).

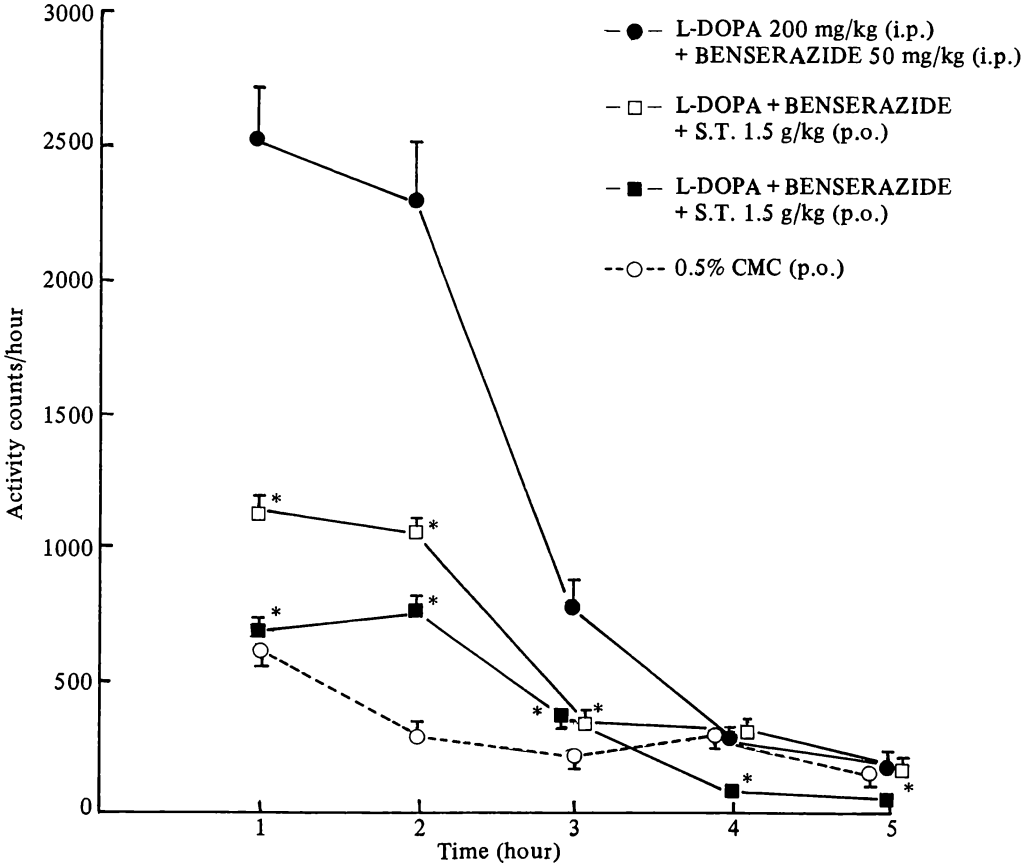

Fig. 3. Effects of Suanzaorentang on changes in locomotor activity produced by levodopa plus benserazide in rats. The vertical bars designate the S.E.s'. * $P < 0.05$ (compared to L-DOPA + BENSERAZIDE)

VMA was lowered by 25, 30, and 55% at doses of 1.5, 3.0, and 6.0 g/kg respectively.

HVA was lowered by 31, 35, and 44% at doses of 1.5, 3.0, and 6.0 g/kg respectively.

5-HIAA was lowered by 21, 25, and 49% at doses of 1.5, 3.0, and 6.0 g/kg respectively.

Discussion

Some Chinese physicians prescribed the ancient Chinese formula of S-T for patients with insomnia. However, up to now there are only 3 scientific reports of this formula. The reports are as follows: Zizyphi semen was reported to have a sedative effect (Watanabe *et al.*, 1973). Ligustici Chuanxiong Rhizoma was reported to have anticonvulsive effect (Kuo, 1980). Ohwada *et al.* (Ohwada *et al.*, 1983) studied the effect of S-T on sleep EEG in adults and found that S-T had no apparent effect on the REM period of sleep.

Previously, we found that S-T could be used in patients with insomnia (Chen and Hsieh, 1985a) and could be a very promising anxiolytic remedy (Chen and Hsieh, 1985b).

We also found that the alcohol-extracted S-T was much more potent in its anxiolytic effect than those of water-extracted S-T and the alcohol extracts of individual components. To understand the mechanism(s) of action of S-T, we conducted the present studies.

We found that S-T could significantly decrease locomotor activity and potentiate hexobarbital-induced sleep. These facts implied that S-T had a sedative effect.

S-T could significantly augment the hypomotility produced by α-MT (an inhibitor of tyrosine hydroxylase) (Widerlov and Lewander, 1978), and reduce the locomotor stimulation produced by levodopa plus benserazide. These facts implied that S-T had effects on central catecholaminergic activity and had antiaggressive and taming effects. Rastogi *et al.* (1977) reported that an agent with antiaggressive and taming effects often had an anxiolytic effect.

The concentrations of central monoamines (NA, DA, and 5-HT) were not significantly altered by S-T but their metabolites (VMA, HVA, and 5-HIAA) were significantly lowered. These facts implied that S-T decreased the turnover of central monoamines.

Pentylenetetrazol- and picrotoxin-induced convulsions were significantly inhibited by S-T but strychnine-induced convulsion was not inhibited by S-T. These facts implied that S-T had anticonvulsive effect but its action might not be at the spinal cord. Whether S-T might be useful on patients with epilepsy deserves further study.

Anticonvulsants may be divided into 3 groups (Iorio *et al.*, 1973): those like dilantin with only anticonvulsive effect; those like phenobarbital with anticonvulsive and sedative effects; and those like diazepam with anticonvulsive, sedative, and anxiolytic effects. Our studies manifested that S-T had anticonvulsive, sedative, and anxiolytic effects. So the effects of S-T on the central nervous system might be similar to those of benzodiazepines. One of our further works is to study the effects of S-T on the serotoninergic system and GABAergic system.

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酸棗仁湯對於大白鼠行爲及中樞單胺濃度之影響

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摘　　要

著者等於前報曾報告我國古方"酸棗仁湯"是一個很好的抗焦慮藥物。爲探討酸棗仁湯之作用機轉，吾等研究其對於大白鼠行爲及中樞單胺濃度之影響。本研究結果發現酸棗仁湯可以有意義地(1)延長－pentylenetetrazol 或 picrotoxin 所引起陣攣性驚厥到強直性驚厥之時間，(2)延長 Hexobarbital 所引起之睡眠時間，(3)減少自發運動量，(4)增加由 α-MT 所引起運動量抑制作用，(5)減少由Levodopa 加 Benserazide 所引起自發運動量之亢進作用，與(6)減少中樞腦內 HVA, VMA 與 5-HIAA 之濃度，但對於中樞腦內 DA，NA 與 5-HT 則無影響。由以上之結果顯示，酸棗仁湯之鎮靜、抗痙攣作用，與降低中樞單胺之轉換速率與兒茶胺系統之活性有關。